Mycobiology 36(2) : 99-101 (2008)

Antifungal Activity of Securinine against Some Plant Pathogenic Fungi

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(Received February 11, 2008. Accepted April 3, 2008)

The alkaloid securinine was assessed against spore germination of some plant pathogenic and saprophytic fungi (*Alternaria alternata*, *Alternaria brassicae*, *Alternaria brassicicola*, *Curvularia lunata*, *Curvularia maculans*, *Curvularia pallenscens*, *Colletotrichum musae*, *Colletotrichum* sp., *Erysiphe pisi*, *Helminthosporium echinoclova*, *Helminthosporium spiciferum*, *Heterosporium* sp.). Spore germinations of all the tested fungi were inhibited. *Alternaria brassicicola*, *C. lunata*, *C. pallenscens* and *H. spiciferum* were highly sensitive as complete inhibition of spore germination was observed at very low concentrations (200 ppm).

KEYWORDS : Antifungal activity, *Phyllanthus amarus*, Securinine, Spore germination

The crude plant extracts and a number of individual isolated principles from plants have been used *in vitro*, in greenhouse and also in open field conditions against several plant pathogenic fungi (Kobayashi *et al.*, 1987; Maillard *et al.*, 1987; Singh *et al.*, 1995; Prithiviraj *et al.*, 1996, 1998; Sarma *et al.*, 1999). The compound ajoene isolated from *Allium sativam* (garlic) and neemazal, a product of *Azadirachta indica* (neem) have been used successively against powdery mildew (*Erysiphe pisi*) of pea under field conditions (Singh *et al.*, 1995; Prithiviraj *et al.*, 1998). Many alkaloids isolated from plants are known to exhibit antifungal activity at very low concentrations (Singh *et al.*, 2000; Maurya *et al.*, 2002). However, the effect of the alkaloid securinine, isolated from *Phyllanthus amarus* on spore germination has not been reported so far. We report here the antifungal activity of this compound for the first time.

The plant *P. amarus* (Family: Euphorbiaceae) is distributed in tropical and sub-tropical regions of the world. The herb is bitter in taste and is reported to possess astringent, deobstruent, stomachic, diuretic, febrifugal and antiseptic properties. It has been reported that *P. amarus* is reasonably safe and effective in acute viral hepatitis specially hepatitis B (Thyagrajan *et al.*, 1988). A number of flavonoids, lignans, triterpenoids, organic acids and alkaloids have earlier been reported in literature (Joshi *et al.*, 1986; Singh *et al.*, 1991; Huang *et al.*, 1992). The antifungal activity of ent-norsecurinine isolated from *P. amarus* has also been reported (Goel *et al.*, 2002).

Materials and Methods

The whole plant of *P. amarus* was collected from Varanasi and dried in sun light. The dried, powdered whole plant (4 kg) was extracted with methanol in a Soxhlet extractor. The methanol extract was dried on water bath and extracted with 7% aqueous citric acid. The acidic fraction was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ fraction was concentrated and chromatographed over SiO_2 gel column eluting with solvents of increasing polarity and each eluted fraction was monitored by thin layer chromatography for their homogeinity. Fractions eluted from $CHCl_3$-methanol (1 : 2) were mixed together and crystallised from methanol which furnished an alkaloid (56 mg), as colourless granules, Rf 0.45 ($CHCl_3$-methanol, 4 : 1), m.p. 140~43°C. It exhibited IR absorption bands at 1840 and 1760 cm^{-1} and UV absorption maxima at 256 nm. Its molecular formula was determined as $C_{13}H_{15}NO_2$ from its molecular ion peak at *m/z* 217 [M^+] in the mass spectrum. The spectral data, *i.e.* ^{1}H-NMR, ^{13}C-NMR and MS were identical with reported data of securinine. Finally, it was identified as securinine

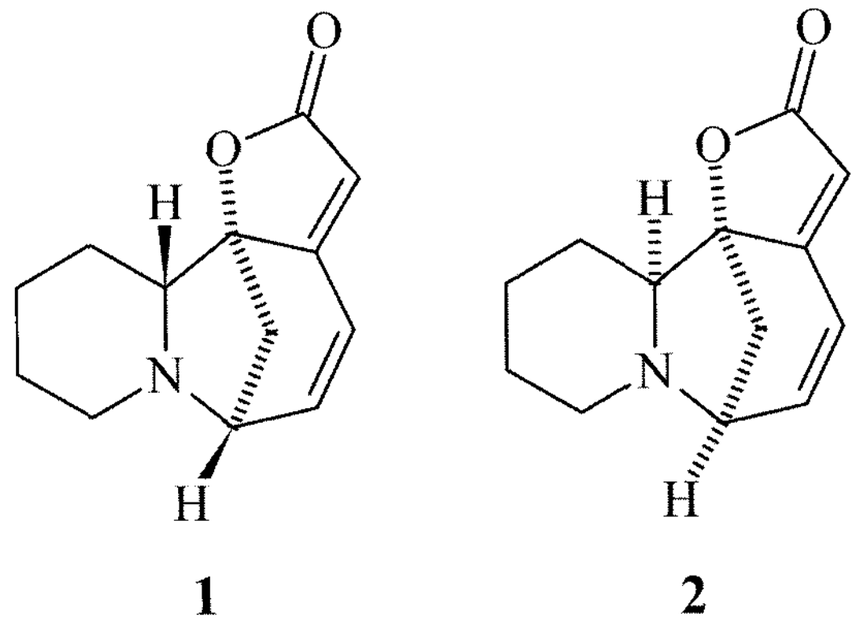

Fig. 1. Structure of securinine and allosecurinine.

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Table 1. Effect of securinine on spore germination of some fungi.

Fungus	Host	Concentration (ppm)					
		Germination rate (%)					
		Control	200	400	600	800	1000
Alternaria alternata	Saprophyte	95.50	88.50	87.17	54.50	50.50	0
Alternaria brassicae	*Brassica compestris*	94.23	91.30	90.50	86.12	90.50	0
Alternaria brassicicola	*Brassica oleracea*	98.80	0	0	0	0	0
Colletotrichum musae	*Musa paradisiaca*	97.60	94.30	55.80	0	0	0
Colletotrichum sp.	*Arundinaria falcata*	94.36	90.50	89.17	86.50	12.50	0
Curvularia lunata	*Oryza sativa*	97.50	0	0	0	0	0
Curvularia maculans	*Musa paradisiaca*	96.60	59.00	36.17	0	0	0
Curvularia pallenscens	*Bambusa indica*	89.15	0	0	0	0	0
Erysiphe pisi	*Pisum sativum*	97.20	80.50	79.50	60.12	40.20	26.30
Helminthosporium echinoclova	*Echinochloa crusgalli*	98.45	86.20	76.12	55.30	36.12	16.23
Helminthosporium spiciferum	*Solanum melongena*	90.12	0	0	0	0	0
Heterosporium sp.	*Casia fistula*	90.68	76.45	60.36	24.68	14.80	0

(**1**) (Fig. 1) (Manske, 1977) by direct comparison with an authentic sample (mmp, co-TLC and superimposable IR).

The fungi (Table 1) were isolated on PDA (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 liter) from their respective hosts collected from the experimental farm of Banaras Hindu University, Varanasi, India. The cultures were purified by single spore isolation technique on PDA slants and maintained by periodic transfer on the same medium for further experiments. Seven to ten days old cultures were used in this experiment. The spores of obligate parasitic fungi were directly picked up from their respective hosts.

The stock solution (2,000 ppm) was prepared by dissolving 10 mg of the alkaloid securinine (**1**) initially with a few drops of methanol in a test tube. After the chemical was completely dissolved, approximately 5 *ml* of distilled water was added. The solvent methanol was then evaporated on water bath. The required concentrations (200, 400, 600, 800 and 1,000 ppm) of the chemical were prepared from the stock solution by diluting with distilled water. A drop (30~40 *ml*) of the chemical solution was placed on a grease free glass slide. Fungal spores (about 200~300 spores) were mixed in a solution with the help of a sterile inoculation needle. *E. pisi* conidia were directly picked up from diseased plants and mixed in a solution. The slides were later placed in moist chamber made by placing two sterile moist filter papers on the lid and base of petri plates. The spores were then incubated at 25 ± 2°C. for 24 hr for germination. The germination of the spores was observed after staining with Cotton blue prepared in lactophenol under a binocular light microscope (Nikon, Japan). All the experiments were conducted in triplicate.

Results and Discussion

The effect of securinine (**1**) on spore germination of some plant pathogenic fungi was studied (Table 1). The sensitivity of different fungi to this chemical varied considerably. *Alternaria brassicicola, Curvularia lunata, Curvularia pallenscens* and *Helminthosporium spiciferum* were the most sensitive as complete inhibition of germination was observed in all the concentrations (200, 400, 600, 800 and 1,000 ppm) of the chemical. Similar effect on *Curvularia maculans* and *Colletotrichum musae* was recorded at 600, 800 and 1,000 ppm. However, only 1,000 ppm was effective against spore germination of *Alternaria alternata, Alternaria brassicae, Colletotrichum* sp. and *Heterosporium* sp. The chemical was mildly effective at all the concentrations studied against *E. pisi* and *Helminthosporium echinoclova*.

A number of alkaloids isolated from plants have already been reported in literature to possesses antifungal activity (Atta-ur-Rahman *et al.*, 1997; Bracher, 1994; Goel *et al.*, 2003).

We have reported the antifungal activity of the alkaloids, ent-norsecurinine (Goel *et al.*, 2002), norsecurinine (Sahni *et al.*, 2005) and allosecurinine (Singh *et al.*, 2007) isolated from *P. amarus*. Securinine (**1**) and allosecurinine (**2**) (Fig. 1) are isomers whereas norsecurinine and ent-norsecurinine have different structures compared to compounds **1** and **2**. Securinine inhibited complete spore germination of fungi *A. brassicicola, C. lunata, C. pallenscens* and *H. spiciferum*, whereas the alkaloid allosecurinine inhibited complete spore germination of fungi *C. lunata, Collectotrichum* sp., *C. musae* and *Heterosporium* sp. at very low concentrations. This is the first report of the antifungal activity of securinine, the fourth alkaloid isolated from *P. amarus*. The efficacy of the chemical is significantly high even at low concentrations which indicates a possibility of its use to control plant diseases under field conditions.

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